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The Agricultural Chemical Society of Japan.

President : Kintaro OHSHIMA.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon. (Part IV).

Carotin and Xanthophyllesters.

By

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(Received May 4, 1932.)

The natural yellow pigments of the domestic cocoons were hitherto identified by the author as chiefly consisted of xanthophylls, from which crystallizable pigment was isolated and identified as lutein (leaf xanthophyll).

In this research the occurrence of other natural yellow pigments such as carotin and xanthophyllesters, which might be possible as in the case of xanthophyll to be derived from the mulberry leaves, is searched and attained the following results.

1) Xanthophyllesters such as physalien, Helenien do not exist in the yellow cocoons neither in golden yellow nor in orange yellow species, though the occurrence of xanthophylls in the mulberry leaves may be assumed in a part as such esters.

2) The fact, that carotin of the yellow cocoons does not exist in the pure crystallizable but partially oxidized state, is verified by the spectrometric method, using as control the purely crystallized carotin of carrots and its partially oxidized one.

3) The carotin content of the yellow cocoons shows 0.6～0.8 mg. per 100 g. air dried cocoon layers: these figures show from one-fortieth to one-seventieth of the xanthophyll content.

4) As the carotin fractions of both golden yellow and orange yellow cocoons are the same regarding their coloring tones in appearance and their modes of absorption bands of the visible part, different pigments can't be supposed to exist in this fraction. The origin of the different coloring tones of both species must be asked in another fractions.

On the Nutritive Value of Pentosan. III.

The Glycogen Accumulation in the Body of Rats by the Xylan Feeding.

By

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(From the Laboratory of Biochemistry, Morioka Imperial College of Agriculture and Forestry.)

(Received May 30, 1932.)

In a previous communication of this investigation[2] it was concluded that the total amount of glycogen accumulated in the body of rabbit, when xylan was administered, is nearly equal to that accumulated by the administration of the same amount of starch. In this paper a brief report is made on the effect of xylan feeding upon the glycogen content in the albino rat, which were selected as an example of omnivorous animals.

Experimental.

Young albino rats were divided into three groups, one of them was subjected to a fasting test, the other to a xylan feeding test, and the latter to a starch feeding test as a collateral. The detail of the experimental procedure was nearly equal as described in the second report of this investigation. The results obtained in ragard to both the glycogen content and blood constituents, which were also studied in this connection, may be shown in Table I, II, III.

Table I. The fasting test.

Date. (1930~1932)	'30. Oct. 1	Oct. 28	Oct. 31	'31. Jan. 29	Jan. 30
Sex of albino rat.	male	male	female	female	female
Hours fasted.	25.5	24.0	24.0	24.5	24.0
Body weight, g.	208	180	105	133	149
Liver weight, g.	8.4	10.5	4.5	7.0	7.8
Liver glycogen, mg. in 100 mg.	11	37	32	60	41
Muscle weight (calculated), g.	97.8	84.6	49.4	62.5	70.0
Muscle glycogen, mg. in 100 g.	21	25	105	274	118
Total glycogen, in 100 g. body.	10.3	14.0	50.6	132.0	57.6
Do. average.	12.2		80.1		
Total blood sugar, mg. in 100 c.c.	—	—	106	96	98
Nonfermentable sugar. "	—	—	—	—	33
Nonprotein nitrogen. "	—	—	—	54	38
Haemoglobin. %	—	—	17.1	17.3	17.2

Table II. The xylan feeding test.

Date (1930~1932).	1930	1930	1931	1932	1930	1931	1931
	9, 20	10, 1	3, 21	4, 18	10, 3	1, 29	1, 30
Sex of albino rat.	m.	f.	f.	f.	f.	f.	f.
Hours fasted.	23.5	20.0	24.0	22.0	9.0	8.0	8.0
Hours, fed to killed.	2.0	3.5	2.0	2.0	16.0	18.0	16.0
Hours, fasting begun to killed.	25.5	23.5	26.0	24.0	25.0	26.0	24.0
Body weight, g.	152	115	180	145	94	134	145
Xylan eaten, g.	2.0	2.4	2.0	1.9	3.0	2.5	2.4
Liver weight, g.	6.2	5.0	7.1	7.0	3.7	7.0	7.2
Liver glycogen, mg. in 100 g.	15	46	57	61	64	128	115
Muscle weight (calculated), g.	71.4	54.1	84.6	68.2	44.2	63.0	68.2
Muscle glycogen, mg. in 100 g.	16	173	165	171	158	256	148
Total glycogen in 100 g. body.	8.1	83.3	79.8	83.4	76.8	127.0	75.2
Do. average.	8.1		82.2			93.0	
Do. increase compared with fast.	−4.1		2.1			12.9	
Do. increase per 1 g. xylan.	−3.1		1.4			5.8	
Total blood sugar mg. in 100 c.c.	—	100	98	90	86	104	95
Nonfermentable blood sugar. "	—	—	34	—	—	36	32
Nonprotein nitrogen. "	—	—	40	38	—	49	38
Haemoglobin. %	—	—	17.9	16.9	17.9	16.9	17.4
Xylan recovered from dig. tract.	—	—	1.83	1.50	—	—	1.45

Table III. The starch feeding test.

Date. (1930~1932)	'30. Sep. 20	'32. Mar. 29	Apr. 18.	'30. Oct. 31	'31. Jan. 31
Sex of albino rat.	male	female	female	female	female
Hours fasted.	24	24	22	9	8
Hours, fed to killed.	2	2	2.5	16	16
Hours, fasting begun to killed.	26	26	24.5	25	24
Body weight, g.	184	175	140	111	143
Starch eaten, g.	4	4	4	4	2
Liver weight, g.	7.8	7.2	7.2	4.7	7.1
Liver glycogen, mg. in 100 g.	1060	1394	1470	1284	214
Muscle weight (calculated), g.	86.5	82.3	65.8	52.2	67.2
Muscle glocogen, mg. in 100 g.	452	391	264	247	94
Total glycogen in 100 g. body.	257.4	241.3	199.3	170.5	54.8
Do. average.	257.4	220.3		112.7	
Do. increase compared with fast.	245.2	140.2		32.6	
Do. increase per 1 g. starch.	113.0	54.6		13.0	
Total blood sugar, mg. in 100 c.c.	—	109	112	115	103
Nonfermentable blood sugar. "	—	32	—	—	31

Nonprotein nitrogen, mg. in 100c.c.	—	40	39	45	39
Haemoglobin, %	17.4	17.1	16.8	17.2	16.9
Starch recovered from dig. tract.	—	2.07	1.95	—	0.3

The increase in the amount of glycogen by xylan feeding is found to be very small when compared with that in the case of starch feeding. The amount of absorbed xylan too is much smaller than that of absorbed starch. This, by the way, is a fact never noticed in the case of rabbits as was communicated in the first and second report[1][2]. Therefore, sueh a comparison should be based on the amount of substances absorbed. The digestibility of xylan two hours after the feeding was found to be 14.6%, and that of starch 49.8%. The digestibilities 16 hours after the feeding were not estimated then. Ordinary digestibility coefficients were, therefore, estimated afterwards, detail accounts of which will be reported in the next paper, but the results are briefly as follows: xylan 33.1%, starch 86.2%. These digestibility coefficients may be applied, for the comparison without any appreciable error, to the case of 16 hours after the feeding. Thus the increase in the amount of glycogen per gram of digested xylan or starch may be shown in Table IV, and figure.

Table IV. The glycogen increase per gram of digested xylan.

	Xylan feeding.			Starch feeding.		
	one ♂	av. three ♀	av. three ♀	one ♂	av. two ♀	av. two ♀
Hours after the feeding.	2.0	2.5	16.6	2.0	2.3	16.0
Glycogen increase mg.	neg.	96	18	227	127	15

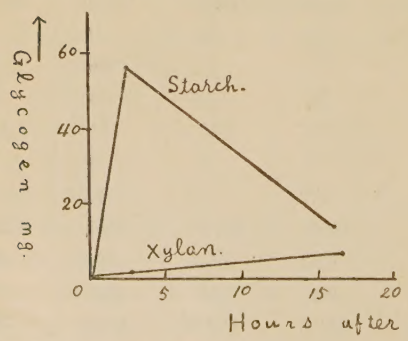

Fig. I. Glycogen increase per gram of eaten material.

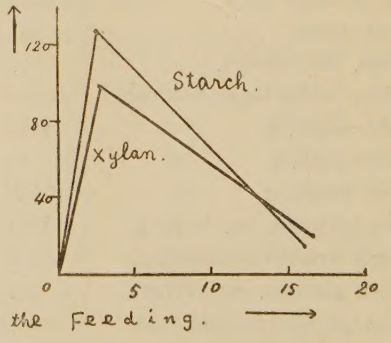

Fig. II. Glycogen increase per gram of digested material.

Conclusion.

1. Glycogen could be accumulated in the body of albino rat by the

feeding of xylan. The accumulation, however, is very small in comparison with the application of starch. This may be attributed mainly to the difficulty in the digestion of xylan by rats. Accordingly, the increase in the amount of glycogen per gram of digested xylan is never so much small in comparison with that caused by the starch.

2. So far as the amounts of nonfermentable sugar, nonprotein nitrogen and haemoglobin are concerned, there was no appreciable difference noticed in the blood of rats, when examined at the end of either 2.5 or 16 hours after the feeding of xylan or starch or none of them. The total blood sugar, however, was found decreased somewhat in the case of xylan.

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(2) Do. Do. Do. p. 33 (1931).

On the Nutritive Value of Pentosan. IV.

The Methods of Treatments and the Kinds of Animals as two of the Effecting Factors upon the Digestibility of Pentosan.

By

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(Received May 30, 1930.)

The effect of various treatments, such as disintegration with alkaline solutions etc., upon the digestibility of pentosan was studied, using various gramineae straws which were recently applied to the investigation of straw-disintegration by the author[1-5]. The results obtained are shown in Table I. The effects upon the digestibility of crude fiber and nitrogen free extract may be added here for a reference.

Table I.

The effect of various treatments upon the digestibility of pentosan.

Materials and treatments.	Digestibility of pentosan by sheep av. %	Ratio of digestibility (untreated, as 100)		
		Pentosan	Crude fiber	N. free ext.
Rice straw, untreated.	52.6	100	100	100
Do. soaked 4 hours in 0.25% NaOH	53.7	102	101	87
Do. soaked 4 hours in 0.75% NaOH	82.9	158	141	153
Do. soaked 4 hours in 1.5% NaOH	82.1	156	139	156

Do. boiled 3 hours in water.	58.6	111	102	105
Do. boiled 3 hours in 1% CaO	87.9	167	141	139
Do. boiled 1.5 hours in 1% CaO	82.8	157	131	139
Do. soaked 2 days in 1% CaO	79.5	151	130	131
Do. soaked 2 days in wood ash extract.	63.5	121	110	119
Barley straw, untreated.	43.8	100	100	100
Do. boiled 3 hours in 1% CaO	74.8	171	128	125
Do. boiled 1.5 hours in 1% CaO	77.6	177	132	118
Wheat straw, untreated.	56.2	100	100	100
Do. boiled 3 hours in 1% CaO	76.8	137	124	121
Do. soaked 4 hours in 0.9% NaOH	75.8	135	125	129
Coarse hay of "Susuki", untreated	34.8	100	100	100
Do. boiled 2 hours in 1% CaO	72.0	207	156	156
Straw of "Hiye", untreated.	43.9	100	100	100
Do. boiled 2 hours in 1% CaO	80.1	182	154	113

Next, the difference in digestibility of pentosan among rabbits,[6][7] albino rats[8] and guinea pigs was studied. The xylan, isolated from rice straw, was used as an example of pentosans. Experiments were carried out in an usual way, namely, a certain amount of the xylan with basal fodder was fed to each animal. The results obtained are summarised in Table II.

Table II.

The digestibilities of pentosan by rabbits, rats and guinea pig.

	Digestibility of xylan.	Digestibility of starch.
Two rabbits, average %	78.9	83.6
Two albino rats, av. %	33.1	86.2
One guinea pig. %	62.9	—

Conclusion.

1. When the straw of rice, barley, wheat, hiye (*Panicum Crus-Galli*, L. var. frumentaceum, Hook.) or coarse hay of susuki (*Miscanthus sinensis*, Anders.) was either soaked 4 hours in a cold solution of 0.75～1.5% caustic soda, or two days in 1% lime suspension, or boiled 1.5～3 hours in 1% lime, the digestibility of pentosan was found to have become 1.4～2.1 times as that of the untreated material. When the rice straw, however, was either soaked 4 hours in 0.25% caustic soda solution, or two days in wood ash solution, or boiled 3 hours in water, no increase in the digestibility of pentosan was noticed.

2. The increase in digestibility of pentosan was found somewhat greater

than that of crude fiber, or of nitrogen free extract, yet bearling nearly a constant ratio between them.

3. The digestibility of xylan, isolated from rice straw, varied remarkably as the kind of animals used. The rabbit digested 78.9% of xylan, the albino rat 33.1% of it, and the guinea pig 62.9% of it. In the case of potato starch, however, the rabbit digested 83.6%, and the albino rat 86.2% of it.

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(5) Do. : J. Scientific Agr. Soc., No. 327, 93 (1931).
(6) Do. : This journal, Vol. 6, 804 (1930).
(7) Do. : Do. Vol. 7, 583 (1931).
(8) Do. : Do. Vol. 8, 90 (1932).

MISSPRINT CORRECTED.

1. First report of this investigation (this journal. Vol. 7, p. 35, 1931.).
   14th line; 3029 calories, should be corrected to read as, 2552 calories.
2. Second report (this journal. Vol. 7, p. 41, 1931).
   8~9th line; by 0.1%, 8~10 hours, should be corrected to read as, by 0.01%, 8~18 hours.

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On the Alcoholic Fermentation of the Amino-acids. Part I.

By

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(Received June 7, 1932.)

It has been shown by F. Ehrlich in a series of famous researches that the alcohols contained in fusel oil come from the amino-acids which are formed by the hydrolysis of the proteins. But little is known about the fermentation products derived from some of the simpler amino-acids, especially of the normal type. Also it has not yet been clear about the origin of normal propyl alcohol though it is thought to be one of the usual constituents of fusel oil.

The author found with vanilline-H_2SO_4 reagent[1] that there was some

production of fusel oil in the fermented, modified Hayduck solution in which several amino-acids were used in place of asparagine; the quantity of produced fusel oil was ca. 0.03~0.1% when glycocoll, *d*-alanine, *d-l*-alanine, *d-l*-α-amino-*n*-butyric acid, *l*-leucine, *d-l*-leucine, *d-l*-valine and *d-l-n*-valine were used, while trace in cases of glutamic acid, asparagine, ammonium-phosphate, ammonium-sulphate, yeast-juice and sugar alone.

Vigorous fermentations were observed in cases of alanine, leucine, Am.-phosphate, Am.-sulphate, glutamic acid and asparagine, while glycocoll and valine were proved to be unadequate nitrogen nutriments for yeast, the last substance being the most inferior. The less the yeast inoculated is, the more fusel oil is obtained. Thus it is supposed that if the yeast is too much inoculated, the sugar may be consumed so quickly that no complete decomposition of amino-acid is expected.

Experimental.

1. Fusel oil produced by saké-yeast in the modified Hayduck solutions in which several amino-acids etc. were used in place of asparagine. Fusel oil was estimated with vanilline-H_2SO_4 reagent colorimetrically.

N-nutriment		Age of culture	Yeast	Products		
				Alcohol	Fusel oil	Total acid
Glycine	0.284%	8 days	1 platinum ear	3.85%	0.07%	0.2006
d-alanine	0.337%	6	〃	4.75	0.07	0.1770
Asparagine	0.25%	6	〃	4.85	0.02	0.1652
Glutamic acid-Na	0.64%	6	〃	3.65	0.02	0.2596
Am-phosphate	0.25%	6	〃	2.65	0	0.2360
Am-sulphate	0.5%	6	〃	4.85	0	0.3068
Sugar alone		7	bulk of a pea	1.975	0.003	
r-alanine	0.4%	6	1 platinum ear	5.4	0.10	
r-aminobutyric acid	0.4%	7	3 platinum ears	5.36	0.12	
r-valine	0 35%	18	bulk of a pea	4.5	0.035	
r-n-valine	0.34%	10	〃	5.5	0.020	
l-leucine	0.34%	6	1 platinum ear	5.0	0.08	
r-leucine	0.34%	6	〃	5.0	0.07	
Yeast water*		6	〃	5.0	0	
〃 with cells		6	〃	4.6	0	

* 5 g. of the dried saké-yeast is boiled with 100 c.c. of water for 1 h. and then divided into two parts.

2. The relation between yeast quantity and fusel oil formation.

N-nutriment		Age of culturs	Yeast	Products	
				Alcohol	Fusel oil
r-alanine	0.4%	7	1 platinum ear	4.3%	0.050%
〃	〃	4	3 peas of yeast mud	4.3	0.007
l-leucine	0.5%	7	1 platinum ear	4.3	0.075
〃	〃	4	3 peas of yeast mud	4.4	0.030

(1) M. Yamada: Bull. Agricult. Chem. Soc. Japan, **4**, 33~4 (1928); Chem. Zent., II, 2603 (1928).

On the Alcoholic Fermentation of the Amino-acids. Part II.

Alanine.

By

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(Received June 7, 1932.)

F. Ehrlich obtained *l*-alanine from *r*-alanine when the latter was fermented with the sugar by yeast but did not descrived any volatile products[1]. In that case O. E. Ashdown and J. T. Hewitt perceived that there was some increase of acetaldehyde formation[2] which was now proved to be an ordinary phenomena——that is to say, contrary to their observation the acetaldehyde was not derived from alanine but from alcohol by means of secondary oxidation when yeast came in contact long with the fermented liquid after the alcoholic fermentation[3]. Though the aldehydes such as isobutylaldehyde or isovaleraldehyde have often been found in crude spirit and regarded as the intermediate product from amino-acid to higher alcohol in the so-called alcoholic fermentation of amino-acid, they come actually from the amino-acids by the reaction between amino-acid and sugar in the extractives when heating or distillation is carried out[4]. If alanine be also attacked by yeast according to Ehrlich's view, ethyl alcohol must be the resultant fermentation product. Now the modified Hayduck solution with pure *r*-alanine synthesized from pure ethyl alcohol in place of asparagine was fermented by saké or distillery yeast and the distillate containing some higher alcohols was obtained. Fusel oil fraction which boils over 100° consists mainly of isobutyl-alcohol and the smaller quantity of amyl alcohol. Thus if alanine be the mother substance

of isobutyl alcohol, the substance of four carbon atoms have to come synthetically from the substance of three carbon atoms but the process has not yet been clear. Continued experiments have shown that isobutyl alcohol does never arise from other amino-acids of course containing valine.

Experimental.

1 to 2.5 l. of culture medium in a 2 to 5 liter flask was sterilised as usual by being boiled for 1 h. every three days and then yeast was added. After about 10 days the fermentation was over and the whole sugar was decomposed. Yeast was filtered and the clear filtrate was first distilled in the pot-still of about 15 liters. The distillate then redistilled through the fractional columb with 5 bulbs several times until the last distillate showed no fusel oil reaction with vanilline-H_2SO_4 reagent. The residues of each distillation except the first were collected and redistilled. Yellow oily layer and the ether extract of water layer were mixed, which was then fractionated in a small flask of about 30 c.c. The first distilling residue containing nonvolatile substances was evaporated on the water bath and filtered. Then from the greenish clear filtrate copper was removed with H_2S phosphate with ammonia and the filtrate was evaporated into syrup to which 90% of alcohol was added and was allowed to stand in a cold place for a day. Crystals of amino-acid was disolved in a hot water, decolorized and recrystallized. The result of the fractionation of oil is as follows.

Remarks :-

I, III, V Modified Hayduck solution with alanine in place of asparagine.
II Sugar alone (8%) without nitrogenous substances and mineral matters.
IV Sugar and alanine without mineral matters.
V One platinum ear of yeast is propagated in 100 c.c. of medium which is poured into the 1 liter of medium. The latter is divided among ca. 30 liters.

	I	II	III	IV	V
Yeast inoculated	Sacch. saké No. 1 10 g. as dry yeast	Sacch. saké No. 1 63 g.	Sacch. saké No. 1 27.6 g.	Beer yeast 5575 g. (wet)	Distillery yeast from 1 pt. ear
Alanine	*d*; 0.4%	——	*r*; 0.34%	*r*; 0.4%	*r*; 0.34%
Total medium	30 L.	20 L.	37.56 L.	50 L.	32.2 L.
Period of ferment.	12 days	7	10~12	6	10~12
Temp.	26~8°	27.9°	24~9°	14°	26~8°
Alcohol produced	——	4.0%	4.7%	4.85%	4.5%
Total acid	0.1298%	0.0944%	0.0912	0.0413	0.1062
Aldehyde	0.00241%	——	0.00164	——	0.00453
Fusel oil	0.05%	0.005%	0.07	0.007	0.1
Alcohol (yield)	1410 c.c. (94%)	695 c.c. (94.2%)	1735	3720 (93.5%)	1510 (95%)

Alcohol (yield) (fusel oil)	41 c.c. (3.57 g.)	47 (91.6%) (0.14 g.)	16.5 (1.65 g.)	44 (0.7 g.)	67 (5.9 g.)
Oil	5.35 g.	0.6	6.1	1.8	9.8
Fract. distill. of oil					
100~106°	0.65 g.	0	0.5	0	1.4
106~113°	2.1 (I B)	0	2.3 (III B)	0	4.6 (V B)
113~122°	0.3	0	0.3	0.15	1.8
122~128°	0.6	0.2 (II A)	0.8 (III A)	0.3	0.8 (V A)
128~130°	1.1 (I A)	0.3	0.9	1.0	1.0
Residue	0.25	—	0.5	0.3	0.2
Yield of yeast (dry)		54 g.	110	1650	55
Alanine recovered	30 g.	—	45.6	149	45.5
Rotation (in 20% HCl)			$[\alpha]_D^{22°} = 6.00°$		$[\alpha]_D^{21°} = 6.43°$

Identification of the fractions.

The following derivatives of alcohol were prepared.

Phenylcarbamate :–

Fraction	M. P.	Analysis				
		Subst. g.	N (c.c.)	T.	P. (m.m.)	N (%) found
I B	80°	0.0983	6.2	15°	768	7.46
III B	83°	0.0798	4.9	13	765.5	7.33
V B	83°	0.0854	5.4	18	758	7.31
Isobutyl alcohol	80°					7.25 ($C_{11}H_{15}O_2N$)

3 • 5 • dinitrobenzoate :–

Fraction	M. P.	Analysis				
		Subst. g.	N (c.c.)	T.	P. (m.m.)	N (%) found
I B	84°	0.0536	4.75	16°	764	10.41
I A	57°					
II A	52°					
III B	86°	0.0511	4.55	15	761	10.47
III A	62°	0.0582	4.90	15	762.5	9.92
V B	85°	0.0753	6.85	19	761.2	10.51
V A	61°	0.0525	4.55	22	756	9.79
Isobutyl alcohol	87°					10.45 ($C_{11}H_{12}O_6N_2$)
Isoamyl alcohol	62°					9.93 ($C_{12}H_{14}O_6N_2$)

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(4) M. Yamada: Bull. Agricult. Chem. Soc. Japan, **4**, 18~21 (1928); Chem. Zent., II, 585 (1929); Bull. Agricult. Chem. Soc. Japan, **4**, 89~91 (1928); also see
S. Akabori: Proc. Imp. Acad. Tokyo, **3**, 672~4 (1927); Chem. Zent., I, 1757 (1928).

On the Alcoholic Fermentation of the Amino-acid. Part III.

α–amino–*n*–butyric acid.

By

Masakazu YAMADA.

(Imp. Brew. Experimental Station, Takinogawa machi near Tokyo.)

(Received June 7, 1932.)

Abderhalden, Chang and Wurm obtained *l*–isomeride as the decomposition product by yeast from *r*–amino–*n*–butyric acid and supposed that *d*–component was the naturally occurring isomeride. But then they did not pay attention to the volatile products. According to Ehrlich's view *n*–propyl-alcohol ought to be produced from the amino acid.

The author's experiment showed that the fusel oil fraction produced by saké yeast in the modified Hayduck solution with this amino acid in place of asparagine consisted mainly of an amyl alcohol which boiled at about 123~8°. From the rotatory power of the alcohol and the properties of two derivatives, this was identified as active amyl alcohol.

Experimental.

d–l–α–amino–*n*–butyric acid was prepared from *n*–propyl alcohol merck.

Medium: Cane sugar 100 g., amino acid 3 g., Hayduck mineral solution 20 c.c., water 980 c.c.

1 to 2.5 l. of culture medium in a 2 to 5 l. flask was sterilized in ordinary way and then saké yeast was added—23 g. as dry yeast for total 13.2 l. of the medium. After 11 days at 25~9° the fermentation was over and the fermented liquid contained no sugar. The distillation and the treatment of the residue are the same as the case of alanine (see part II).

	Alcohol	Total acid	Fusel oil
Fermentation products	4.4%	0.1416%	0.07%
Yield of alcohol	632 c.c. (93%)	24 (90%) (fusel oil 0.768 g.)	oil (B. P. over 100°) 7.3 g.

Fractionation of the oil.

	B. P.	Yield (g.)		B. P.	Yield (g.)
I	100~107°	0.2	V	126~127.5°	1.7
II	107~116°	0.3	VI	127.5~128.5	2.0
III	116~123°	0.1	VII	residue	0.5
IV	123~126°	1.7			

Identification of the fractions.

The following derivatives of alcohol were prepared.

Phenyl carbamate :–

Fract.	M. P.	Analysis				
		Subst. (g.)	N (c.c.)	T.	P. (m.m.)	N (%) found
IV	32.5°	0.0715	4.2	15°	765	6.93
Act. amyl alcohol	30°					6.76 ($C_{12}H_{17}O_2N$)

3–5–dinitrobenzoate :–

Fract.	M. P.	Analysis				
		Subst. (g.)	N (c.c.)	T.	P. (m.m.)	N (%) found
IV	80°	0.0564	4.7	15°	768.3	9.89
V	81.5	0.0666	5.7	15	760.5	10.05
Amyl alcohol						9.93 ($C_{12}H_{14}O_6N_2$)

Rotation of alcohol (mixture of IV, V, VI).

$[\alpha]_D^{23°} = -5.55°$ $r = -3.62°$ $l = 2.2$ dm. $c = 29.64\%$

$[\alpha]_D^{20°} = -5.90°$ (methyl ethyl carbin carbinol).

Amino acid recovered from the distilling residue was 7.6 g. and its rotation in 20% HCl solution was following.

$[\alpha]_D^{22°} = -1.14°$ $r = -0.16°$ $l = 2.2$ dm. $c = 6.37\%$

Chemical Studies of Agar-Agar.

III. On the Isolation of free λ acid from Hydrato-Kanten-λ by means of Electric Dialysis.

By

E. Takahashi and K. Shirahama.

(Received July 4th, 1932)

λ acid (Kanten-acid), a principal constituent of agar-agar and its constitution is denoted by $R{\cdot}O{\cdot}SO_2{\cdot}OH$, was isolated from the hydrato-kanten-λ* (obtained by hot water-hydrolysis of agar-agar) by means of electric dialysis using Pauli's apparatus.

Two methods were adopted for the isolation of this acid.

(1) After treating the dialyzed solution with small quantity of ammonium chloride solution, pourd into 99% alcohol (4～5 volumes of original solution) by which λ acid was precipitated as a ammonium salt.

(2) After adding to the dialyzed solution 4 volumes of 99% alcohol, the same volume of ether, and further 2.5 times as much benzol as total volume. The mixture was condensed to a small volume at 40～50°C under diminished pressure.

Next, 3～4 volumes of ether, was added to the solution by which free λ acid was settled as a white voluminous precipitate.

General natures of various preparations are summarised as Table I : —

Summary.

(1) Aqueous solution of λ acid gives colloid. This acid is stable in the combined form with alkalis or metals. But in the free state, it is unstable and easily decomposable.

(2) The rotately power of this acid shows −31° and its composition as far as made clear is as follows : —

Galactose	39.47%	Pentose	8.17%
SO_4	ca. 8.00%		

Besides above, it contains a substance giving ketose reaction and a hexose any other than galactose. The presence of uronic acid and methylpentose is excluded.

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* Previous report. This J. 8 659, (1932).

Table I.

No.	Preparation						Ash %	CO_2 %	$[\alpha]_D$	SO_4 %	Titration acidity for 100 g. Sample	Others
	Concentration of dialysed solution		Strength of Electric Current			Treatment						
	λ g.	Water c.c.	Volt.	m.Amp.	Hour.							
1							6.27	0.76	——	6.26	acidic	Galactose=40.65% Pentose=3.40%
2	10	200	110	2.5	48	NH_4Cl+alcohol	5.85	1.16	——	——	——	Ca=2.65% SO_4=3.02%
3	5	200	110	10	24	NH_4Cl+alcohol	3.68	0.80	——	——	——	
4	15	200	110	50~100	30	NH_4Cl+alcohol	——	——	——	8.36	——	Galactose=39.47% Pentose=8.17%
5	10	200	110	50~100	30	NH_4Cl+alcohol	0.80	0.56	−29.4°	8.72	NaOH=1.56g. (SO_4=3.18 g.)	Ammonia N=0.81% (SO_4=5.55%)
6	10	200	110	50~100	30	After neutralised with NH_4OH NH_4Cl+alcohol	0.77	——	——	7.66	NaOH=0.36g. (SO_4=0.86 g.)	Ammonia N=0.90% (SO_4=6.17%)
7	10	200	110	50~100	30	After neutralised with $Ba(OH)_2$ alcohol	——	——	——	8.66	——	SO_4 to be combined with Ba=0.138%
8	10	200	110	50~100	35	NH_4Cl+alcohol	Trace	——	−31.0°	6.90	NaOH=1.05g. (SO_4=2.52 g.)	Ammonia N=1.27% (SO_4=4.36%)
9	10	200	110	50~100	30	Isolation by mixed solvent	0.59	——	−30.8°	6.62	NaOH=3.13g. (SO_4=7.52 g.)	

Qualitative reaction: —— Reducing power negative; ketose, hexose and pentose positive; methylpentose and uronic acid negative.

On the Formation of Lysolecithin from Egg-yolk Lecithin by Pancreas Extract.

By

Ziro Nikuni.

(Received July, 11th., 1932.)

Since the discovery of lysolecithin by Delezenne and Fourneau (Bull. Soc. Chim. iv. **15**, 421, 1914) many works have been done on this subject and it is now generally recognized that the formation of this substance from lecithin by the action of snake venom is due to an enzyme "Lecithinase" in it.

On the other hand, the existence of an enzyme which splits lecithin into fatty acids, glycerophosphoric acid and cholin, was found by Bókay (H. 1, 157, 1877) in cow pancreas. This enzyme seems to be widely distributed in blood, liver, brain und other organs, as well as in egg-yolk, castor bean and Takadiastase etc. So the question naturally arises whether it is the same with the lecithinase of snake venom, or whether lysolecithin is also formed from lecithin during the digestion with this enzyme.

Contardi and Latzer (Bioch. Z. **197**, 222, 1928) obtained a haemolytic substance from the decomposition products of lecithin by Ricinus lipase. Wohlgemuth (Bioch. Z. **39**, 302, 1912) found a haemolytic substance in human pancreatic juice, and Belfanti (Bioch. Z. **154**, 148, 1924: Z. f. Imm. **44**, 347, 1925; **56**, 449, 1928) isolated lysolecithin from pancreas and salivary glands of horse and cow, and supposed that the latter might be formed by the enzyme contained in these organs, but no decisive proof has been given on this subject.

According to the sugestion of Prof. U. Suzuki, the present author tried to digest egg-yolk with pancreas extract or with commercial pancreatin, and observed that a powerful haemolytic substance was formed during the digestion, which disappeared again on further standing. After many trials, the author succeeded in isolating this substance in crystalline state and proved it to be identical with lysolecithin, formed by snake venom. It was further observed that a haemolytic substance is also formed by commercial Takadiastase in the same way.

Experimental.

(1) Digestion of egg-yolk lecithin with pancreas extract.

(a) The pancreas extract containing lecithinase was prepared according

to the method of Willstätter (H. **125**, 153, 1923). The finely crushed pig pancreas was, after being treated with acetone and ether respectively to remove water and fatty substances, dried in a vacuum desiccator. The brown powder thus obtained was now macerated with 16 volumes of 80% glycerin for 4 hours at 30°, then filtered, and the brown filtrate containing the enzyme was used for the experiment.

(b) Sometimes, the finely minced pancreas paste was directly used for the same purpose.

(c) Fresh egg-yolk was mixed with a N/15 potassium-phosphate solution and digested with the pancreas extract, prepared as mentioned above, at 36～38°, being added with a little toluol. The mixture was frequently shaken, and from time to time a definite amount was taken out from it and added with 95% alcohol. The precipitate formed thereby was filtered off, and the filtrate was evaporated nearly to dryness and treated with a small amount of absolute alcohol, filtered, and to the filtrate an alcoholic solution of cadmium chloride was added, whereby the lysolecithin together with lecithin were precipitated as the double salts. These were collected, dried, and again dissolved in 0.9% NaCl solution and tested for haemolytic power according to the Hirao's method (J. Agr. Chem. Soc. Japan, **6**, 738, 1930), using 2% suspension of red blood corpuscles. The experiment was repeated several times with nearly the same result. For example, one experiment is given below.

Experiment: as the source of lecithinase, either 5 g. of pancreas paste, or 12 g. of the glycerin extract obtained from 5 g. pancreas paste, were used. 5 fresh egg-yolks (85 g.) were well mixed with N/15 potassium-phosphate solution, and filled up to 300 c.c. Each 100 c.c. of this mixture was now treated as follows.

1. Control: 100 c.c. yolk solution and 3 c.c. toluol.
2. Pancreas paste: 100 c.c. yolk solution and 3 c.c. toluol and 5 g. paste.
3. Glycerin extract: 100 c.c. yolk solution and 3 c.c. toluol and 12 g. extract.

The digestion was carried out at 36～38°. Each 5 c.c. was taken out from time to time, and tested for haemolytic power as descrived above.

The results are given in the following table.

Table I.

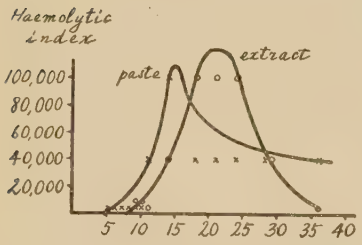
	1	2	3	4	5	6	7	8	9	10
Hours:	15min.	3 hrs.	20 hrs.	2 days	3 days	4 days	5 days	6 days	7 days	8 days
Control:	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace
Paste:	trace	400	trace	400	400	400	4,000	4,000	4,000	4,000
Extract:	trace	trace	trace	trace	trace	400	400	400	400	400

	11	12	13	14	15	16	17	18	19
Hours:	9 days	10 days	11 days	14 days	17 days	21 days	24 days	29 days	36 days
Control:	trace	trace	trace	trace	400	400	400	trace	
Paste:	4,000	4,000	40,000	100,000	40,000	40,000	40,000	40,000	40,000
Extract:	4,000	4,000	4,000	40,000	100,000	100,000	100,000	40,000	4,000

The numbers of the table indicate the haemolytic index (Kofler: Die Saponine, 148, 1927). For instance, the number 40,000 shows that the 1/40,000 solution of the sample to be tested causes the complete haemlysis.

The results are shown graphically in Fig 1.

Fig. 1.



The results show that, during the digestion of egg-yolk with pancreas extract, a substance of strong haemolytic power is formed. The highest activity being attained after 10～20 days. After that time, it decreases rapidly and finally disappears.

(2) Digestion of egg-yolk lecithin with commercial pancreatin.

(a) The above experiment was repeated with commercial pancreatin, prepared by Konishi & Co. according to Japanese Pharmacopoea in the following way : fresh pig or cow pancreas is finely crushed and macerated with two parts of water, saturated with chloroform. After standing for 12 hours it is filtered from insoluble residue, the oparescent filtrate is once more filtered and evaporated to dryness at 45°. In this way a yellowish white powder having a characteristic smell and flesh-like taste is obtained. It is completly soluble in water, but insoluble in alcohol.

(b) 5 yolks were mixed with N/15 potassium-phosphate solution and filled up to 300 c.c. For the experiment, 100 c.c. of this solution were digested with 1/2 g. pancreatin, being added with 3 c.c. toluol. At the same time, another 100 c.c. of the solution were treated with viper venom, obtained from one viper (Agkistrodon blomhoffii) under the same condition. The digestion was carried out at 36～38° and the haemolytic power was determined with the following results.

Table 2.

	1	2	3	4	5	6	7
Hours:	5 min.	2 hrs.	19 hrs.	2 days.	3 days.	4 days.	6 days.
Viper venom:	40,000	40,000	40,000	40,000	100,000	100,000	100,000
Pancreatin:	0	40,000	40,000	40,000	100,000	200,000	200,000

	8	9	10	11	12	13	14
Hours:	8 days.	10 days.	13 days.	16 days.	21 days.	28 days.	39 days.
Viper venom	100,000	60,000	50,000	40,000	40,000	4,000	4,000
Pancreatin	200,000	100,000	100,000	100,000	80,000	100,000	100,000

This results show that the haemolytic substance of high activity was formed during the digestion. The activity reaches the maximum in 5～6 days and afterwards decreases rapidly. It is also noteworthy that the curves of pancreatin and viper venom in figure 2 show remarkable similality.

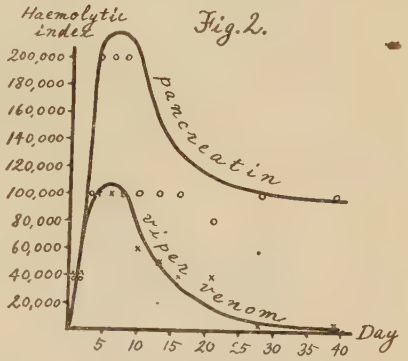


Fig. 2.

(3) Isolation of lysolecithin from the digestion liquid of egg-yolk with pancreatin.

The method adopted in this experiment was essentially the same with that of Mr. Iwata (J. Agr. Chem. Soc. Japan, **6**, 759, 1930: **7**, 557, 1931; Bioch. Z., **224**, 430, 1930).

30 fresh yolks (515 g.) were mixed with 500 c.c. of N/15 potassium-phosphate solution and treated with 5 g. commercial pancreatin. The mixture was kept at 36～38° for 2½ hours with frequent shaking, no antiseptic being used in this case. It was then poured into an equal volume of warm 95% alcohol, filtered, the residue was washed twice with each 400 c.c. 85% alcohol. Both filtrate and washings were united and evaporated in vacuum until about 80 gramms thick brown syrup was obtained. This syrup was again dissolved in 200 c.c. absolute alcohol and filtered, the insoluble residue was washed three times with each 20 c.c. absolute alcohol. The combined filtrate thus obtained was now added with 15 g. cadmium chloride, dissolved in 250 c.c. hot absolute alcohol, whereby a white voluminous precipitate was formed. After addition of 300 c.c. acetone, the cadmium precipitate was filtered, washed five times with a mixture of alcohol and acetone, and dried in a vacuum desiccator. A white powder was thus obtained. The yield was 31 g. and the haemolytic index about 60,000.

The cadmium double salts, obtained as above was now suspended in a mixture of 100 c.c. chloroform and 50 c.c. of absolute alcohol, and decomposed by adding 80 c.c. of 4% alcoholic ammonia. The precipitated cadmium hydroxide was centrifuged off and the clear filtrate was evaporated to dryness, treated with 50 c.c. absolute alcohol, filtered from insoluble residue and the clear brown filtrate was poured into 1½ litre ether, whereby the crude

lysolecithin was precipitated: yield $5\frac{1}{2}$ g.

This crude product was recrystallized from absolute alcohol, alcohol and chloroform, pyridin, chloroform, alcohol and acetone and finally from alcohol and chloroform. In this way the lysolecithin was obtained as colourless nice prisms. Yield $\frac{1}{2}$ g.

The lysolecithin, purified as above, is slightly hygroscopic. Heated in a sealed capillary, it begins to soften at 95° and decomposes to a brown liquid at 262～263°. The haemolytic index is 320,000, Mixed with the lysolecithin prepared by Mr. Iwata, using cobra venom, no depression of decomposition point was observed. The analysis gave the following results.

Sample	Method	C%	H%	P%	N%	Amino N / Total N
(1) 4.188 mg.	Liebig's	58.24	10.26			
(2) 4.119 mg.	"	58.16	10.23			
(3) 5.144 mg.	Lieb's			5.95		
(4) 4.680 mg.	Dumas'				3.00	
(5) 5.941 mg.	"				2.97	
(6) 10.308 mg.	van Slyke's					3/100
mean		58.20	10.24	5.95	2.98	3/100
theory ($C_{24}H_{50}PNO_7$)		58.12	10.17	6.26	2.82	0/100

Summary.

When egg-yolk lecithin was digested with pancreas extract or commercial pancreatin, a powerful haemolytic substance was formed as the intermediate product.

This substance was isolated in pure crystalline state, and was proved to be lysolecithin. It was identical in every respect with lysolecithin formed from lecithin by the action of snake venom.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind guidance throughout this work.

(June 20th, 1932, Agr. Chem. Lab. Tokyo Imperial University, Komaba)

The Quantitative Studies of some Chemical Constituents of the Mulberry Leaves. Part I.

The Study of the Cystine- and Cysteine-Compounds in the Mulberry Leaves.

By

Yukitaro Kishi.

(Received August 9 th, 1932).

Résumé.

(1) For the purpose of studing mulberry culture, I made a quantitative study of the cystine- and cysteine-compounds in the mulberry leaves, using Okuda's S. S. A. method[1].

(2) Mulberry trees are so vigorous in growth that, even if the stems are cut off close at the stub twice or thrice in succession in a year, new buds form more readily and the leaves and stems thus produced grow more vigorously and luxuriantly than in the case of many other trees similarly treated ; therefore, the quantity of the cystine- and cysteine-compounds in the fresh matter of the leaves is small in the buds and very young leaves, but increases in proportion to the growth of the leaves, and reaches the maximum quantity at the part where the action of the synthesis is most vigorous, and then decreases again in the fresh matter of the old leaves. In these compounds in the mulberry leaves, the SH form is found present in greater quantity than the S–S form at each stage of the growth of the leaves.

(3) The quantitative difference of the cystine- and cysteine-compounds contained in the leaves depends on the varieties of the mulberry trees ; this is especially so in the young leaves. In the leaves of "Roso", the amount of these compounds tends to be smaller than in the leaves of other varieties ; this is especially so in the young leaves used to feed young silkworms.

(4) My experiment has shown that a "hishage" stem (without leaves) of a mulberry tree contains a greater quantity of the cystine- and cysteine-compounds than a healthy stem (without leaves) ; while, on the contrary, the leaves of the "hishage" stem contains a smaller quantity of these compounds than the leaves of the healthy stem.

The "hishage" is an epithet given by many Japanese sericulturists to a

(1) Journal of the Department of Agriculture, Kyûshû Imperial University, Vol. 2, No. 5, (1929) ; also Journal of the Agricultural Chemical Society of Japan, **3**, 1907, (1927).

physiologically unsound stem. The "hishage" stem is flattened as if crushed, and is fatter in the affected parts than a healthy stem.

Addition: — In studying the amount of the cystine- and cysteine-compounds contained in the mulberry leaves, I have also examined the amount of these compounds contained in a silkworm made to abstain from food. I found that the quantity of the S–S form increases gradually, contrary to that of the SH form.

The Quantitative Studies of some Chemical Constituents of the Mulberry Leaves. Part II.

The Acid–base Balance of the Ash in the Mulberry Leaves.

By

Yukitaro Kishi.

(Received August 9 th, 1932).

Résumé.

(1) I made a study of the acid–base balance of the ash in the mulberry leaves.

(2) The total alkalinity of the ash in the mulberry leaves was determined after incineration, with or without the addition of magnesium nitrate. In either instance, the alkalinity increased in proportion to the growth of the mulberry leaves, as the contents of the ash in the leaves increase in proportion to the growth.

(3) The total alkalinity of the ash in the mulberry leaves treated with magnesium nitrate is generally a little less than that obtained by the ordinary method.

(4) The alkalinity of the ash which is soluble in water decreased in proportion to the growth of the mulberry leaves, as against the total alkalinity in the two instances mentioned above, which increased in proportion to the growth. Consequently, the alkaline ash constituents which is insoluble in water showed an increase in proportion to the growth of the leaves.

Isolation of "Oryzanin" (Antineuritic Vitamin) from Rice-polishings. (Second Report.).

By

Sator Ohdake.

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(Received August 10 th, 1932).

In the previous report[1], the author stated that the antineuritic substance was isolated in crystalline state as hydrochloride from rice-polishings and the molecular formula $C_6H_8N_2O_2$ was assigned to this substance, the formula being different from that given by Jansen and Donath[2].

During the studies on sulphur compounds of yeast extract, U. Suzuki and the author[3] found that the antineuritic preparation, "crude oryzanin", obtained from rice-polishings as well as from yeast-extract gave the reaction of sulphur and observed that the antineuritic activity was always accompanied by the sulphur reaction. Reminding this fact, the author detected the presence of sulphur in the active hydrochloride refered to above and the molecular formula given in the first report had to be corrected as $C_{12}H_{16}N_4SO_2$–2HCl.[4]

A. Windaus and his coworkers[5] reported, recently, that they isolated the antineuritic vitamin in the form of picrolonate from yeast extract. They stated that the antineuritic yeast vitamin contains sulphur which is detectable by boiling with a strong alkali solution in the presence of lead acetate and the active substance should be designated by the formula $C_{12}H_{17}N_3SO$ by analysing the picrolonate. Although the analytical results do not agree with each other it is presumed, from chemical properties, that the antineuritic yeast vitamin isolated by Windaus would be identical with oryzanin isolated from rice-polishings by the present author. Van Veen[6] in Java also reported that

(1) The proceeding of the Imperial Academy of Japan, **7** (1931), No. 3, 102~105 ; J. Agr. Chem. Soc. Japan, **7** (1931), 775~808.

(2) B. C. P. Jansen and W. F. Donath: Mededeeling van den Dinst der Volksgezondheid in Ned-Indie. Anno. (1927), Part I, 1~15.

(3) U. Suzuki, S. Ohdake u. T. Mori: Biochem. Z. **154**, (1924), 280; J. of Agr. Chem. Soc. of Japan. **1** (1924), 1~10.

(4) Bul. of Agri. Chem. Soc. Japan, **8**. 1~3 (1932), 11~46.

(5) A. Windaus, R. Tschesche, H. Ruhkopf, F. Laquer. & F. Schultz: Zeit. fur physiol. Chem., **204** 3~4, (1932), 123~128.

(6) A. G. Van Veen: Zeit. fur physiol. Chem., **208** (1932), 125~128.

he found the existence of sulphur in the antineuritic hydrochloride isolated from rice-polishings and corrected his previous formula as $C_{12}H_{20}N_4SO_2$–2HCl which resembles closely with that given by the present author.

In order to confirm the composition of the active substance, the author prepared, further, sufficient quantity of the active hydrochloride in pure state and compared it, to the affirmative effect, with the preparations purified through picrolonate and chloraurate. Besides the hydrochloride ($C_{12}H_{16}N_4SO_2$–2HCl) the author prepared the picrolonate ($C_{12}H_{16}N_4SO_2$–$2C_{10}H_8N_4O_5$), the picrate ($C_{12}H_{16}N_4SO_2$–$2C_6H_3N_3O_7$), the chloraurate ($C_{12}H_{16}N_4SO_2$–$2HAuCl_4$), and the chlorplatinate ($C_{12}H_{16}N_4SO_2$–H_2PtCl_6) and found always that the base has the composition of $C_{12}H_{16}N_4SO_2$.

Experimental.

The preparation of the active hydrochloride: — In the present work, so-called "Oryzanin-extract", an antineuritic concentrate of rice-polishings was fractionated directly by silver nitrate and baryta to simplify the previous process. The silver precipitates, obtained at the Pн. 4.5～6.8 was decomposed with dilute hydrochloric acid and it was treated successively with phosphotungstic acid, alcohol, and platinum chloride in alcohol and finally the hydro chloride was crystallized out from alcoholic solution by adding acetone as described in the previous paper. The crude crystals of the hydrochloride were purified by repeating the recrystallisation from alcohol and acetone, untill the crystals showed the constant melting point and revealed to be quite uniform under the polarisation microscope. The yield: 1.6 g. from 11.500 kg. of original rice-polishings.

The hydrochloride prepared in this way possessed entirely the same properties with that purified further through picrolonate and chloraurate. The analytical results also agreed with the formula $C_{12}H_{16}N_4SO_2$–2HCl given in the previous work.

The properties of the hydrochloride: — The purified hydrochloride crystallises in colorless long plates (Fig 1), melting with decomposition at 249～250°C (uncorr.). It is readily soluble in water, sparingly in alcohol, but insoluble in acetone, ether and benzene etc.

The aqueous solution of the hydrochloride gives precipitates with phosphotungstic acid, mercuric chloride, silver nitrate and baryta, picrolonic acid, platinum chloride, gold chloride, iodine potassium iodide and Dragendorff's reagent, but gives no precipitate with picric acid, lead acetate, tannic acid, mercuric sulphate or with flavianic acid. It gives sulphur reaction which is detectable by giving a violet coloration with sodium nitroprusside or by giving black precipitates with lead acetate when it is boiled with alkali or fused

with metallic sodium previously, while the substance itself gives no reaction with these reagents or with barium chloride in the aqueous solution itself.

It gives Pauly's diazo-reaction, though the coloration is quite different comparing with that given by histidine, histamine or thymine. When it is added with the reagent, it gives yellowish coloration for few minutes which turns to red gradually. It gives also a weak greenish coloration by ferriferricyanide reagent or by phosphomolybdic acid and ammonia while the ninhydrin reaction, purin-reactions, i. e. Kossel's, Weidel's and xanthin-reactions, as well as the arginine reaction by α-naphtol and sodium hypochlorate are all negative.

Rotatory power was measured with 30.995 mg. of the hydrochloride in 1.50887 g. (26°C) of water but no rotation has been observed.

Ultraviolet absorptions spectrogram was taken with mol/10.000. aqeous solution of the hydrochloride, the absorption maxima was exhibited at 270 mμ. (the broad band extending 280～260 m μ.) and 239 m μ. (extending 230～244 m μ.)[7] (Fig. 7). Irradiation destroyed the activity of the hydrochloride.

Analysis of the hydrochloride : —

No.	Subst mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	S%	Cl%
(A) M.Pt. 250°C (Lot No. 1～5.)								
(1)	4.230	6.286	2.090	40.53	5.49	—	—	—
(2)	4.383	6.455	2.194	40.17	5.56	—	—	—
(3)	2.859	0.379 c.c. N (16°C 755 mm.)		—		15.55	—	—
(4)	2.734	0.365 c.c. N (15°C 757 mm.)		—		15.75	—	—
(5)	4.311	3.718 mg. AgCl		—	—	—	—	21.32
(6)	5.374	3.614 mg. $BaSO_4$		—	—	—	9.23	—
(B) M.Pt. 249°C (Lot. No. 6.)								
(7)	5.412	8.225	2.714	41.44	5.57	—	—	—
(8)	5.235	7.949	2.471	41.41	5.25	—	—	—
(9)	5.194	7.837	2.656	41.15	5.68	—	—	—
(10)	4.385	0.604 c.c. N (15°C 757 mm.)		—		16.26	—	—
(11)	4.915	0.676 c.c. N (12°C 752 mm.)		—		16.29	—	—
(12)	4.287	0.578 c.c. N (15.5°C 756 mm.)		—		15.87	—	—
(13)	5.499	4.413 mg AgCl		—	—	—	—	19.84
(14)	5.261	4.236 mg AgCl		—	—	—	—	19.91
(15)	6.031	3.996 mg $BaSO_4$		—	—	—	9.10	—
(16)	5.668	3.758 mg $BaSO_4$		—	—	—	9.11	—
(C) M.Pt. 250°C (Lot No. 7)								

(7) The spectrogram was taken by Dr. M. Sumi with Hilger's quartz spectrograph ; Cf. Guha : Biochem. J. **25** (1931), 941 ; A. Windaus & his co-workers : Zeit. Physiol. Chem. **204** (1932), 127.

(17)	5.068	7.602	2.463	40.99	5.40	—	—	—
(18)	5.145	7.765	2.573	41.16	5.56	—	—	—
(19)	4.253	0.598 c.c. N (23°C 752 mm.)		—		16.04	—	—
(20)	4.342	0.617 c.c. N (25°C 752 mm.)		—		16.10	—	—
(21)	4.879	0.674 c.c. N (23°C 753 mm.)		—		15.78	—	—
(22)	4.995	3.363 mg. $BaSO_4$		—	—	—	9.25	—
(23)	5.942	3.958 mg. $BaSO_4$		—	—	—	9.15	—
(24)	6.016	4.930 mg. AgCl		—	—	—	—	20.26
(25)	5.513	4.489 mg. AgCl		—	—	—	—	20.13
(D) M.Pt. 249~250°C "Purified through Picrolonate".								
(26)	4.355	6.491	2.177	40.65	5.55	—	—	—
(27)	4.863	7.224	2.458	40.51	5.62	—	—	—
(28)	4.270	6.418	2.166	40.99	5.64	—	—	—
(29)	4.504	0.637 c.c. N (25°C 749 mm.)		—		15.96	—	—
(30)	4.600	0.647 c.c. N (24°C 751 mm.)		—		15.97	—	—
(31)	4.194	0.578 c.c. N (23°C 753 mm.)		—		15.74	—	—
(32)	5.078	3.469 mg. $BaSO_4$		—	—	—	9.38	—
(33)	5.364	3.643 mg. $BaSO_4$		—	—	—	9.33	—
(34)	4.834	3.907 mg. AgCl		—	—	—	—	19.98
(35)	5.002	4.055 mg. AgCl		—	—	—	—	20.04
(E) M.Pt. 250° "Purified through chloraurate"								
(36)	5.526	8.302	2.768	40.97	5.53	—	—	—
(37)	3.160	0.451 c.c. N (25°C 752 mm.)		—		16.17	—	—
(38)	5.077	3.490 mg. $BaSO_4$		—	—	—	9.44	—
(39)	4.210	3.428 mg. AgCl		—	—	—	—	20.11
Cal. for $C_{12}H_{16}N_4SO_2$–2HCl				40.79	5.10	15.86	9.07	20.11
Cal. for $C_{12}H_{18}N_4SO_2$–2HCl				40.56	5.63	15.78	9.01	20.00

These results agree most closely with $C_{12}H_{16}N_4SO_2$–2HCl.

The activity of oryzanin hydrochloride: — The antineuritic acitivity of the hydrochloride was tested on pigeons and albino rats with the following results: — (1) To pigeons suffering from polyneuritis by exclusive feeding on polished rice, 0.008~0.01 mg. of the hydrochloride were injected daily. The symptoms were improved in a few hours and the pigeons cured from polyneuritis soundly in 1~2 days. (Chart. 1.)

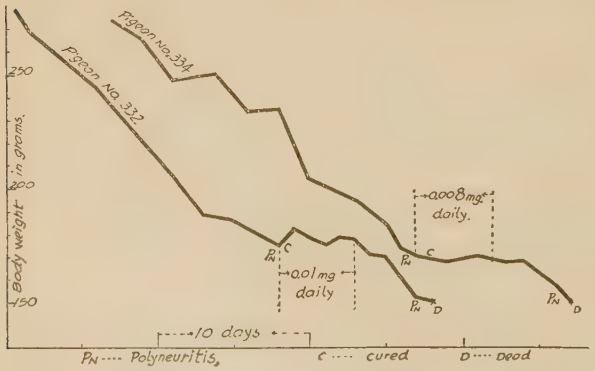


Chart. 1—Pigeons on polished rice & Oryzanin hydrochloride.

(2) Pigeons fed on the artificial diet[8], consisting of 60% purified starch, 20% purified casein, 15%

(8) Chick and Roscoe: Bioch. J. **22.** (1928), 790.

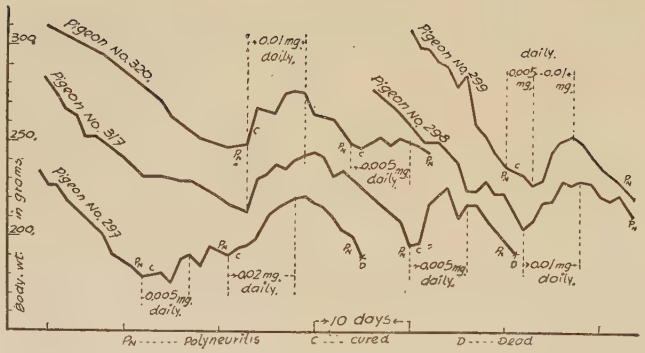


Chart. 2—Pigeons on artificial diet & Oryzanin-hydrochloride.

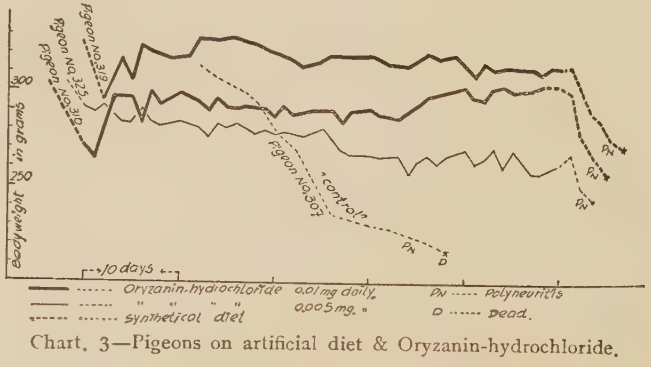


Chart. 3—Pigeons on artificial diet & Oryzanin-hydrochloride.

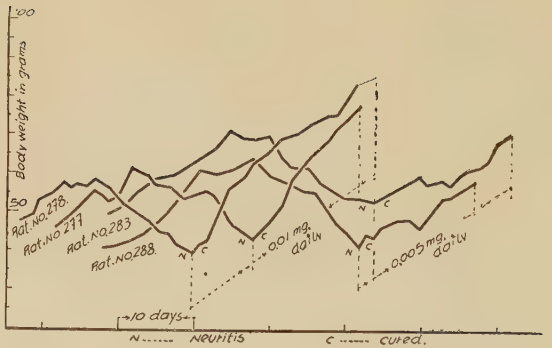


Chart. 4—Rats on artificial diet & Oryzanin-hydrochloride.

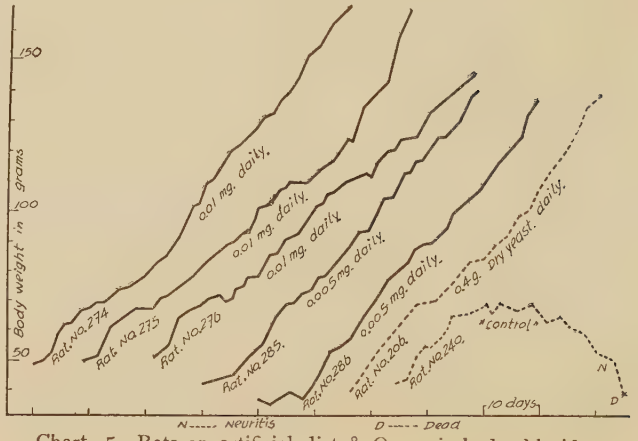


Chart. 5—Rats on artificial diet & Oryzanin-hydrochloride.

peanuts oil and 5% McCollums salt mixture, supplemented with 0.4 g. of autoclaved yeast daily, exhibited the typical symptoms of polyneuritis after 3 weeks usually. By injecting 0.005～0.01 mg. of the hydrochloride daily, the main symptoms were improved in 2～3 hours and cured in 1～2 days. (Chart. 2).

(3) Pigeons fed on the same artificial diet and administered daily with 0.005～0.01 mg. of the hydrochloride in aqueous solution per os, remained in perfect health for 50 days. (Chart. 3).

(4) Young rats, about 40～50 g. in weight, fed on the same artificial diet, supplemented with 3 drops of cod liver oil and 0.4 g. of autoclaved yeast daily, exhibited the symptoms of neuritis in 5 weeks commonly. By supplementing with 0.005—0.01 mg. of the hydrochloride daily, they were cured quickly and their growth curves paralleled almost to the normal. (Chart. 4).

(5) Young rats, fed on the same diet as above, but supplemented daily with 0.005～0.01 mg. of the hydrochloride from the begining of the experiment, grew normally with perfect health for 60 days. (Chart. 5).

(6) The curative day-dose[9] for a pigeon was found to be of the order of 0.0025 mg. as shown in the following table.

Pigeon. No.	Body-weight g.	Days to polyneuritis	Body-Weight suffering from polyneuritis	Dose injected mg.	Days of protection	Day-dose [Dose/Days, protected] mg.	
333	342	22	191	0.012	4	0.0030	
310 (i)	321	24	202	0.01	3	0.0033	
310 (ii)	321	27	199	0.01	3	0.0033	
331	289	22	177	0.01	5	0.0020	
332	285	18	179	0.01	4	0.0025	
334	274	18	185	0.01	5	0.0020	
311	328	34	181	0.008	3	0.0027	Average 0.0025
313 (i)	304	32	172	0.008	3	0.0027	
313 (ii)	304	35	176	0.008	3	0.0027	
324 (i)	342	26	205	0.008	4	0.0020	
324 (ii)	342	30	194	0.008	4	0.0020	
326	317	27	205	0.008	3	0.0027	
330	291	28	184	0.005	3	0.0017	

The picrolonate of oryzanin : —

When the alcoholic solution of picrolonic acid was added to the aqueous solution of the hydrochloride, the light yellow picrolonate separated out which was collected and recrystallised from hot dilute alcohol. It crystallises in

(9) Kinnersly and Peters: Bioch. J. **19** (1925), 820.

light yellow needles (Fig. II) or prisms (Fig. III) and melts sharply at 226°C with decomposition evolving gas. It is readily soluble in hot alcohol, sparingly in water but insoluble in ether, benzene etc. Dried at 100°C in vacuum and analysed.

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	S%	Picrolonic acid %
(1)	5.882	10.270	2.266	47.62	4.28	—	—	—
(2)	5.145	8.989	1.950	47.65	4.21	—	—	—
(3)	4.955	0.848 c.c. N (15°C 755 mm.)		—		20.15	—	—
(4)	4.919	0.857 c.c. N (16°C 756 mm.)		—		20.47	—	—
(5)	4.382	0.764 c.c. N (15°C 752 mm.)		—		20.45	—	—
(6)	8.505	2.263 mg. $BaSO_4$		—	—	—	3.64	—
(7)	8.520	2.320 mg. $BaSO_4$		—	—	—	3.74	—
(8)	141.5	92.7 mg. Picrolonic acide.		—	—	—	—	65.51
(9)	161.7	106.7 mg. "	"	—	—	—	—	65.99
Cal. for $C_{12}H_{16}N_4SO_2$-$2C_{10}H_8N_4O_5$				**47.53**	**3.96**	**20.79**	**3.96**	**65.53**
Cal. for $C_{12}H_{18}N_4SO_2$-$2C_{10}H_8N_4O_5$				**47.48**	**4.20**	**20.74**	**3.95**	**65.18**
Cal. for $C_{12}H_{17}N_3SO$-$2C_{10}H_8N_4O_5$ (By Windaus & his coworkers)				**49.29**	**4.24**	**19.77**	**4.11**	**67.73**

The picrate of oryzanin : —

The picrate was prepared from the hydrochloride by adding natrium picrate in dilute alcohol. The light yellow picrate sepatated out, was collected and recrystallised from hot dilute alcohol. It crystallises in light yellow plates (Fig. IV), melting with decomposition at 208°C and it is soluble in alcohol, sparingly in water, but insoluble in ether, benzene etc.
Dried at 100°C in vacuum and analysed : —

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	S%
(1)	5.021	7.278	1.375	39.53	3.04	—	—
(2)	5.133	7.441	1.517	39.54	3.28	—	—
(3)	5.197	7.683	1.488	40.03	3.18	—	—
(4)	3.965	0.627 c.c. N (14°C 765 mm.)			—	18.93	—
(5)	4.337	0.701 c.c. N (14°C 764 mm.)			—	19.32	—
(6)	7.850	2.459 mg. $BaSO_4$		—	—	—	4.17
(7)	8.829	2.708 mg. $BaSO_4$		—	—	—	4.21
Cal. for $C_{12}H_{16}N_4SO_2$-$2C_6H_3N_3O_7$				**39.02**	**2.98**	**18.97**	**4.34**
Cal. for $C_{12}H_{18}N_4SO_2$-$2C_6H_3N_3O_7$				**38.92**	**3.24**	**18.92**	**4.32**

The chloraurate of oryzanin : —

The chloraurate crystallises in light orange yellow long plates (Fig. V) melting at 189°C with decomposition. It is soluble in hot water and in

hot alcohol but sparingly in cold, and insoluble in ether, benzene etc.

The analysis of the chloraurate : —

No.	Subst. mg.	CO_2 mg.	H_2O mg.	Au mg.	C%	H%	N%	S%	Au%	Cl%
(1)	7.517	4.000	1.333	3.146	14.51	1.97	—	—	41.85	—
(2)	7.548	4.084	1.336	3.160	14.76	1.97	—	—	41.86	—
(3)	7.814	4.194	1.326	3.240	14.64	1.89	—	—	41.46	—
(4)	5.780	0.255 c.c. N (15°C 751 mm.)			—	—	5.94	—	—	—
(5)	7.355	0.858 c.c. N (15°C 752 mm.)			—	—	5.71	—	—	—
(6)	9.066	2.300 mg. $BaSO_4$		3.784	—	—	—	3.49	41.74	—
(7)	9.434	2.314 mg. $BaSO_4$		3.937	—	—	—	3.37	41.73	—
(8)	4.507	5.409 mg. AgCl		1.884 mg. Au	—	—	—	—	41.80	29.67
(9)	4.542	5.395 mg. AgCl		1.897 mg. Au	—	—	—	—	41.77	29.39
Cal. for $C_{12}H_{16}N_4SO_2$–2HAuCl$_4$					15.00	1.83	5.83	3.33	41.04	29.58
Cal. for $C_{12}H_{18}N_4SO_2$–2HAuCl$_4$					14.97	2.08	5.82	3.33	40.96	29.52

The chlorplatinate of oryzanin : —

Redish orange prisms (Fig. IV) blackening at 245～250°C without melting. It is soluble in hot water but very sparingly in cold, and insoluble in alcohol, ether, benzene etc. Dried at 100°C in vacuum and analysed : —

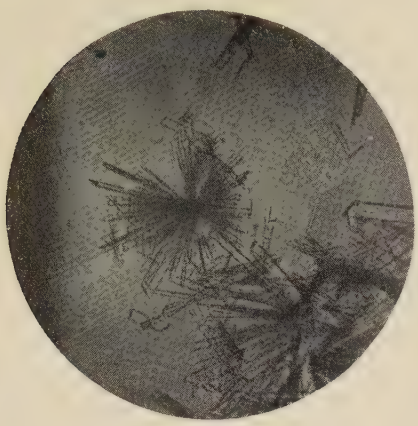
No.	Subst. mg.	CO_2 mg.	H_2O mg.	Pt mg.	C%	H%	N%	S%	Cl%	Pt%
(1)	7.675	5.107	1.555	1.911	20.87	2.59	—	—	—	28.63
(2)	5.471	0.382 c.c. N (14°C 764 mm.)			—	—	8.35	—	—	—
(3)	6.317	0.449 c.c. N (15°C 751 mm.)			—	—	8.32	—	—	—
(4)	5.233	0.3606 c.c. N (16°C 754 mm.)			—	—	8.08	—	—	—
(5)	8.573	2.946 mg. $BaSO_4$		2.439	—	—	—	4.72	—	28.45
(6)	4.496	5.616 mg. AgCl		1.276	—	—	—	—	31.11	28.38
Cal. for $C_{12}H_{16}N_4SO_2$-H_2PtCl_6					20.87	2.61	8.12	4.64	30.87	28.26
Cal. for $C_{12}H_{18}N_4SO_2$-H_2PtCl_6					20.81	2.89	8.09	4.62	30.78	28.18

Free base of oryzanin : —

A hygroscopic amorphous substance, soluble in water, alcohol, methyl-alcohol, and in acetone, but insoluble in ether, benzene etc.

Summary.

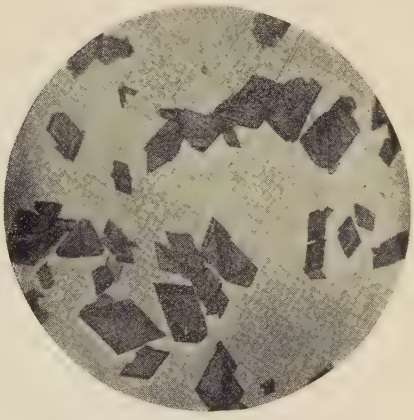
(1) The hydrochloride of oryzanin which was prepared in pure state directly from so-called "Oryzanin-extract" by silver-fractionation, platinum precipitation in alcohol and repeated recrystallisation from alcohol and acetone, possessed entirely the same properties with that purified further through picrolanate and chloraurate. The analytical results agree with the formula



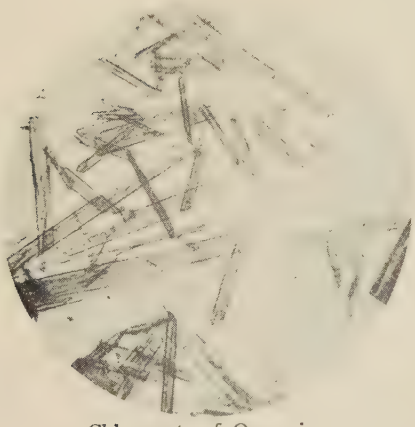
Hydrochloride of Oryzanin.
Leitz. III×2
Fig. I

Picrolonate of Oryzanin, in needles.
Leitz. III×2
Fig II

Picrolonate of Oryzanin, in prisms.
Leitz. III×2
Fig. III



Picrate of Oryzanin.
Leitz. I×2
Fig. IV.



Chloraurate of Oryzanin.
Leitz. I×2
Fig. V.

Chlorplatinate of Oryzanin.
Leitz. III×2
Fig. VI

S. Ohdake: Isolation of "Oryzanin" from Rice-polishings.

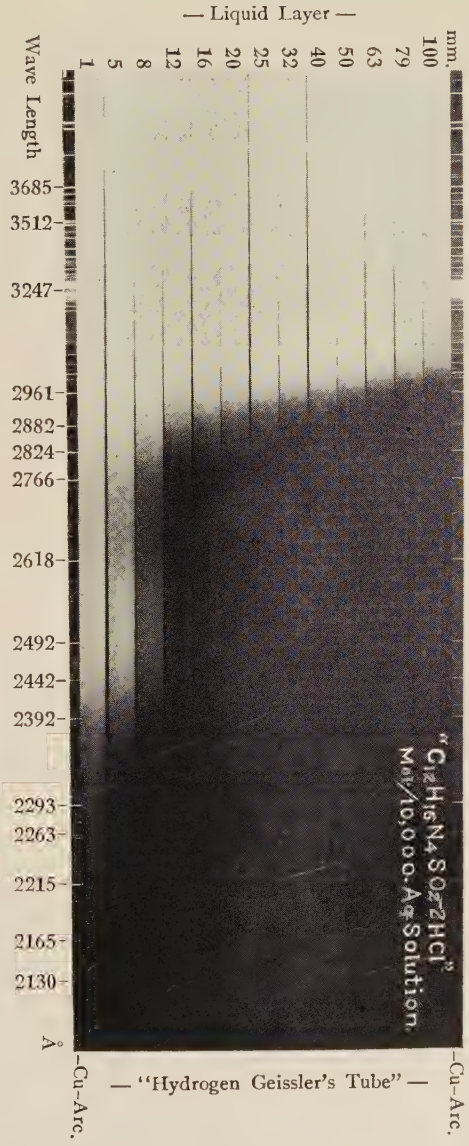


Fig. VII. Ultraviolet Absorptions Spectrogram of Oryzanin Hydrochloride.

$C_{12}H_{16}N_4SO_2$–2HCl which was given by the author in the previous report. The yield: 1.6 g. from 11.500 kg. of rice-polishings.

(2) The hydrochloride cured and protected pigons as well as albino-rats from polyneuritis with the daily dose of 0.005～0.01 mg. The curative day–dose for a pigeon was found to be the order of 0.0025 mg.

(3) Besides the hydrochloride, the author prepared the picrolonate ($C_{12}H_{16}N_4SO_2$–$2C_{10}H_8N_4O_5$ M.Pt. 226°C), the picrate ($C_{12}H_{16}N_4SO_2$–$2C_6H_3N_3O_7$ M.Pt. 208°C), the chloraurate ($C_{12}H_{16}N_4SO_2$–$2HAuCl_4$ M.Pt. 189°C) the chlor-platinate ($C_{12}H_{16}N_4SO_2$–H_2PtCl_6) and finally the free base (amorphous), and studied their properties.

(4) From these results, it was confirmed that the antineuritic substance isolated from rice-polishings by the author, is a new sulphur compound having the empirical formula $C_{12}H_{16}N_4SO_2$.

Further studies on the chemical constitution of this substance will be reported later on.

The author expresses his sincere thanks to Prof. Dr. U. Suzuki for his kind advise and encouragement throughout the work. Thanks are due to Sankyo Company, Ltd. for kind supply of the material. The author is also indebted to Messrs M. Kamada and T. Yamagishi for their assistance both in chemical and biological experiments.

(May 12 th., 1932).

Feeding Experiments with Decomposition Products of Proteins.

By

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(Received July 22, 1932.)

It is now generally assumed that protein in food is in a greater part hydrolysed in the alimentary canal into amino acids and again regenerated to the body protein, specific to each individual organism, so it is very probable that food protein can be substituted by the mixture of amino acids, provided that all necessary amino acids are present in adequate proportion. Abderhalden[1] was the first who tried to settle this problem by feeding experiment. In one experiment with a dog fed on the enzymatic decomposition products of lean meat, prepared by treating with pepsin, trypsin and erepsin successively, he has demonstrated that nitrogen equilibrium was maintained for many weeks. In another experiment with a dog, previously starved and lost a greater part of the body weight, he has observed that by giving the same decomposition products, the animal not only recovered the health but the body weight attained higher than before experiment. In a later period, he gave the mixture of amino acids to the same dog and observed that the nitrogen balance was still maintained, though for a short time.

U. Suzuki[2] reported also the positive results obtained with white rats fed on the synthetic diets containing either pepton or erepton. But he could not obtain any satisfactory results with the acid or baryta hydrolytic products. In this experiments a portion of meat protein was boiled with 25% sulphuric acid for 20 hours, sulphuric acid was carefully removed by baryta and evaporated to dryness. Another portion of meat protein was boiled with saturated baryta solutien for 6 hours and after removing baryta with sulphuric acid, evaporated to dryness. By adding these decomposition products, either alone or in combination, to the extent of 10% of the protein-free synthetic diet, otherwise adequate for growth, the rats could not maintain the health and gradually lost their body weights until finally succumbed.

Mitchell[3], McClendon[4] and Rose[5] have recently reported the results of

(1) Abderhalden: Z. physiol. Chem., **42**~**83** (1904~1913).
(2) U. Suzuki: J. Chem. Soc. Tokyo., **41**, **5** (1920), 381.
(3) Mitchell: J. biol. Chem., **26** (1916), 231.
(4) McClendon: Proc. Soc. Exper. Biol. & Med., **28** (1930~1931), 915.
(5) Rose: J. biol. Chem., **92** (1931), ixvi.

the similar experiments with mice and white rats fed on the synthetic diet containing the mixture of all known amino acids. The rats remained healthy for a month keeping their original body weights, but afterwards gradually declined.

We see thus that further experiments on this subject are desirable. From such a point of view, the present author has carried out some preliminary experiments with white rats, supplying the diets, in which the whole of the protein has been replaced by its partial or complete decomposition products.

In the first place, the enzymatic digestion products, such as Witte's peptone and Teruuchi's peptone were used and these were proved to be capable of replacing the dietary protein completely.

In the next experiments with the sulphuric acid and the baryta hydrolytic products of various degrees it was shown that neither the acid products alone, nor those by baryta could give any satisfactory results, but the mixture of the both was proved to be able to substitute the protein completely.

It is will known that the tryptophane in the protein molecule is completely destroyed by boiling with strong sulphuric acid, but it is not decomposed by baryta, though it undergoes racemization. Further it has been confirmed by several authors that tryptophane is one of the indispensable amino acids for building up the body protein, and it can not be substituted with any other amino acids. Taking these facts in consideration, the auther has carried out further experiments in which the acid hydrolysates were supplemented with tryptophane, instead of the baryta hydrolysates and came to the conclusion that tryptophane is the only factor which is lacking in the acid hydrolysates. By supplying it to the extent of 3% to the latter, the rats could secure the normal growth just like in the case, when the baryta hydrolysates were supplied.

Further experiments with the mixture of purified amino acids are now going on.

Experimental.

I. Experiments with digestion products by enzymes.

Commercial Witte's peptone (the pepsin digest of fibrin) and Teruuchi's peptone (the trypsin digest of casein) were used in this experiments, in the latter case 1% of cystine being added. The both are the mixtures of proteose and peptone, giving a specific biuret reaction. The analysis gave the following results.

	N%	Amino-N%			
		Van Slyke's Method		Formol method	
		Subst. %	N%	Subst. %	N%
Witte's peptone	16.28	1.69	10.39	1.13	6.94
Teruuchi's peptone	13·23	3.81	28.80	4.31	32.58

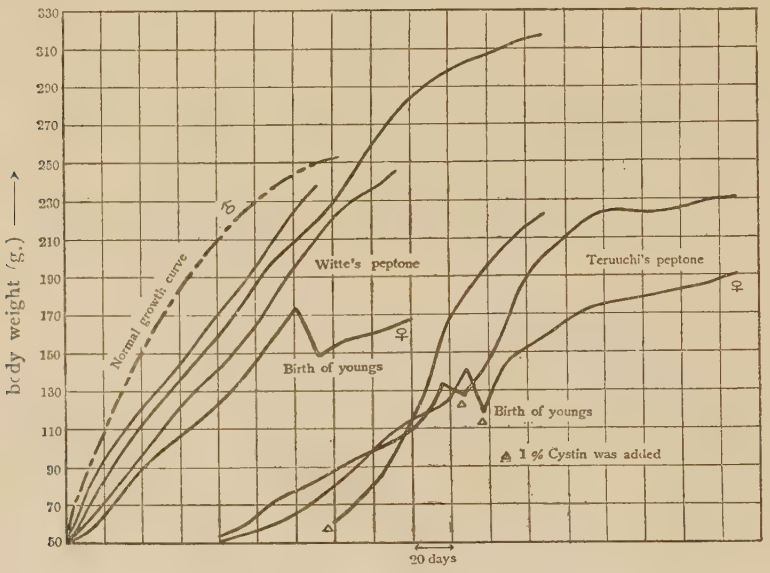


Fig. I.

Young albino rats were fed on the ration consisting of:

Each peptone	10%	Starch	70%
Butter	15%	McCollum's salt mixture	5%
Oryzanin	5 cc per 100 g.		

In both cases the rats grew normally and increased in body weights from 50 ~60 g. to more than 200 g. in 3 months (Fig. I).

II. Experiments with the acid and the baryta hydrolytic products.

(1) Experiments with hydrolytic products of fish meat protein.

The meat protein from codfish (total N 16·11% by Kjeldahl's method) was boiled with 6 times its quantity of 25% sulphuric acid for 30 hours, until it gave no biuret reaction, and showed no more increase of amino-nitrogen when boiled further for an hour. The second and the third portions were hydrolysed partially for one and ten hours respectively, besides the forth one was digested with 70% sulphuric acid at room temperature for 1 week, until

the greater part of the protein was converted into peptone[6].

Another portion of the fish meat protein was boiled with 6 times its quantity of hot saturated baryta* solution for 60 hours. The hydrolysate was free from biuret reaction, and showed no change in amino-nitrogen value when boiled further for one hour. Still other two protions were hydrolysed partially by baryta for one and ten hours respectively.

Sulphuric acid or baryta in each hydrolysed solution was quantitatively removed by baryta or sulphuric acid respectively, each filtrate from barium sulphate was neutralized with dilute sodium hydroxyde solution and then evaporated to dryness at lower tempesature. In each case 85～95 g. of the hydrolytic products were obtained from 100 g. of the protein. The analysis of these products gave the following results.

Condition of decomposition	Biuret reaction	N%	Amino-N %				Rotation		
			Van Slyke's method		Formol method		Subst. g. in 100 c.c. H_2O	α	$[\alpha]_D^{20°}$
			Subst %	N%	Subst %	N%			
by 70% H_2SO_4 at room temp.	+	15.99	4.62	28.93	2.47	15.42	3.2413	−1.65	−50.93
by 25% H_2SO_4 for 1 hr.	+	16.02	7.93	49.48	6.46	40.35	3.0727	−0.68	−22.13
" , " 10 hrs.	−	16.02	11.71	73.11	10.82	67.56	3.2084	+0.31	+ 9.67
" , " 30 hrs.	−	15.95	13.01	83.43	12.91	80.95	2.9435	+0.31	+10.53
by baryta, for 1 hr,	+	14.55	12.16	83.58	12.03	82.68	3.0861	0.00	0.00
" , " 10 hrs.	−	14.43	13.60	94.28	13.33	92.32	3.1411	0.00	0.00
" , " 30 hrs.	−	14.33	14.05	98.00	13.55	94.58	3.6026	0.00	0.00

Millon's, xanthoproteic and Pauly's reaction were positive in all of the above products. Ehrlich's tryptophane reaction by *p*-dimethyl-aminobenzaldehyde was positive with all of the baryta hydrolytic products. With the decomposition products by 70% sulphuric acid and those by 25% sulphuric acid for 1 hour the reaction was slightly positive, while the remaining two acid hydrolytic products gave entirely negative results. Sulphur reaction and the Sakaguchi's reaction for arginine were distinctly positive with the acid hydrolysates, but were entirely negative with the baryta hydrolysate.

Jaffe's rection for amino acid anhydride by picric acid and sodium carbonate were positive, except with the baryta hydrolysate and the 25% sulphuric acid hydrolysate for 30 hours.

From these colour reaction, it can be seen that in the acid hydrolysis,

(6) Fischer & Abderhalden: B., **39** (1906), 752.

* Baryta was recrystallized carefully.

tryptophane was destroyed, while in the baryta hydrolysis, cystine and arginine were decomposed, besides, the presence of amino acid anhydrides, in the partial hydrolytic products by sulphuric acid was indicated. Moreover, it can be seen that the protein loses its optical activity within an hour, when boiled with baryta.

Feeding experiments were carried out with the diets consisting of

Acid hydrolytic products	8%	Butter	15%
Baryta hydrolytic products	8%	McCollum's salt mixture	4%
Starch	65%	Oryzanin	5 c.c. per 100 g.

The combination of the hydrolytic products was as follows:

	Acid hydrolytic products prepared by:		Baryta hydrolytic products prepared by:
I.	70% H_2SO_4 at room temperature	+	Baryta for 1 hour.
II.	25% H_2SO_4 for 1 hour	+	Baryta for 1 hour.
III.	25% H_2SO_4 for 10 hours	+	Baryta for 10 hours.
IV.	25% H_2SO_4 for 30 hours	+	Baryta for 60 hours.

Besides, a diet of the following composition, containing the fish meat protein was used for the control.

Fish meat protein	10%	McCollum's salt mixture	5%
Starch	70%	Oryzanin	5 c.c. per 100 g.
Butter	15%		

Rats fed on the above diets grew healthy and increased in weight from 40 ~50 g. to 130~160 g. in two months, though the rate of growth was much slower than that of the control animals (Fig. II).

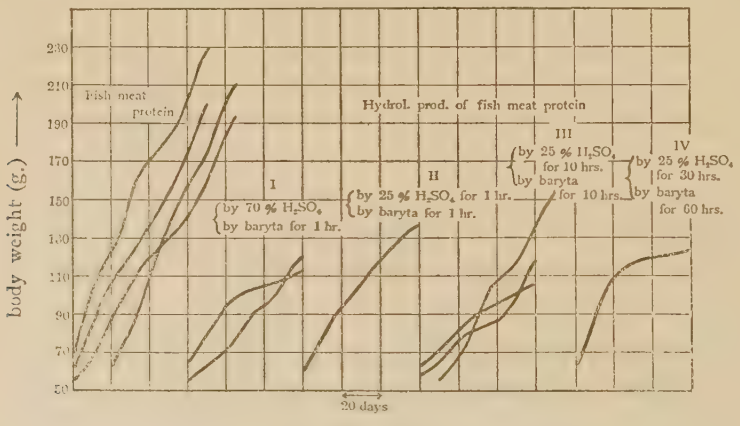


Fig. II.

The fact were worthy of note, that the products hydrolysed by sulphuric acid for 30 hours and those by baryta for 60 hours were free from biuret reaction, and showed, only slight increase in amino-nitrogen value, when boiled further for 5 hours with 20% HCl, as shown in the following example.

(i) The hydrolytic products of fish meat protein (by 25% sulphuric acid for 30 hours), were boiled further for 5 hours with 20% HCl, and the amino-N was estimated as follows:

	Amino-N %			
	Van Slyke's method		Formol method	
	Subst. %	N%	Subst. %	N%
Before boiling with HCl	13.25	83.31	12.91	81.00
After " " "	13.58	85.19	12.91	81.00
Increase	0.33	1.88	0.00	0.00

(ii) The hydrolytic products of fish meat protein (by hot saturated baryta solution for 60 hours), were boiled further for 5 hours with 20% HCl, and analysed as follows:

	Amino-N %			
	Van Slyke's method		Formol method	
	Subst. %	N%	Subst. %	N%
Before boiling with HCl	14.06	98.10	13.47	94.00
After " " "	14.29	99.81	13.75	95.92
Increase	0.23	1.71	0.28	1.92

Feeding experiments were repeated with the mixture of the above hydrolytic products i.e. with the mixture IV. As the source of vitamin B, first oryzanin and then the alcohol extract of yeast were used. The yeast extract was prepared acording to Williams and Watermann[7]. The extract itself or its precipitable part by phosphotungstic acid gave no biuret reaction. 100 c.c. of this extract yielded 12.4875 g. of dry matter and 0.7624 g. of nitrogen, the percentage of nitrogen in the dry matter being 6.11%. No noticeable increase was observed by boiling with 20% HCl for 5 hours more, indicating that almost all of the nitrogen were present in non-peptide form.

	Amino-N %			
	Van Slyke's method		Formol method	
	Subst. %	N%	Subst. %	N%
Before boiling with HCl	3.85	63.00	4.65	76.03
After " " "	3.86	63.24	4.68	76.60
Increase	0.01	0.24	0.03	0.57

(7) Willams & Watermann: J. Biol. Chem., **86** (1930). 275.

Two rats fed on the above diet grew normally from 56 g. and 62 g. up to 330 g. and 230 g. respectively in 8 months (Fig. III, VII, VIII, IX).

(2) Experiments with decomposition products of casein.

Similar experiments were carrid out with the biuret free decomposition products of casein by sulphuric acid and those by baryta. Rats grew from 62 g. and 73 g. to 91 g. and 100 g. respectively in 2 months, though the results were less satisfactory than the preceding experiments (Fig. III).

(3) Experiment with decomposition products of horse meat protein.

To the rats, previously fed on the casein decomposition products for 2 months, the mixture of the biuret-free hydrolytic products of horse meat protein by sulphuric acid and those by baryta were given. Rats grew vigorously as in the case with the hydrolytic products of the fish meat protein, and increased in weight up to 192 g. and 220 g. in 2 months (Fig. III, X, XI).

In the control experiment giving a protein free diet, the rat died within 20～25 days with a remarkable loss of weight. By giving water only without diet, the rats died within 2～3 days (Fig. III). The protein free diet referred to above had the following composition.

Starch	75%	McCollum's salt mixture	5%
Butter*	20%	Oryzanin	5 c.c. per 100 g.
Total N of starch :	0.04%	Total N of buttter :	0.09%

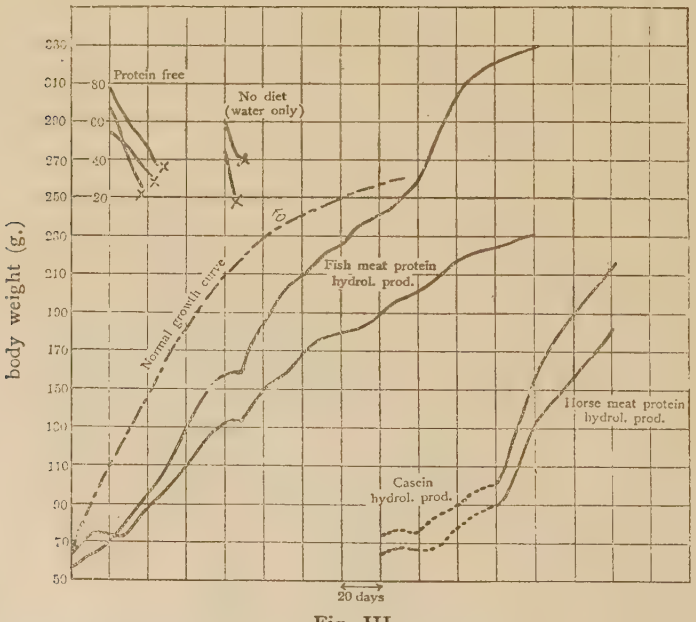


Fig. III.

* The butter used in the experiment was extracted with ether and the ether-soluble portion was evaporated off.

Experiments with decomposition products of fish meat protein.

56 g. 62 g.

Fig. VII, Before the experiment.

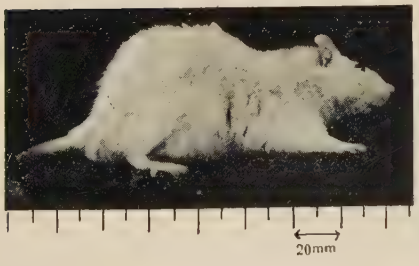
330 g.

Fig. VIII. After 8 months.

231 g.

Fig. IX. After 8 months.

Experiments with decomposition products of horse meat protein.



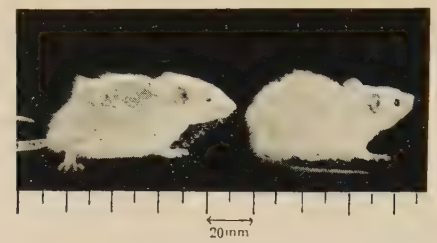
192 g.

Fig. X. After 2 months.

220 g.

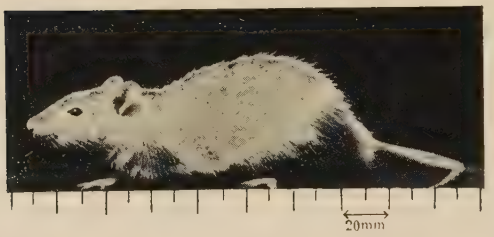
Fig. XI. After 2 months.

Experiments with acid hydrolytic product Supplemented with racemic tryptophane.



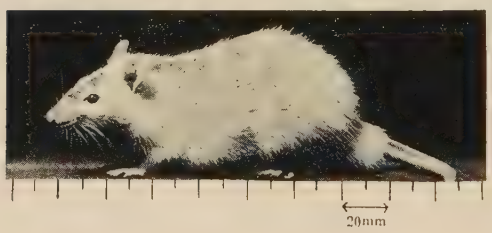
64 g. 65 g.

Fig. XII. Before the experiment.



187 g.

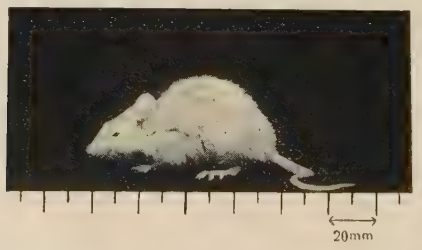
Fig. XIII. After 70 days.



190 g.

Fig. XIV. After 70 days.

Experiment with acid hydrolytic product of fish meat protein.



40 g.

Fig. XV. After 30 days.

III. Experiments with the acid and the baryta hydrolytic products separately.

The acid and the baryta hydrolytic products, complety free from biuret reaction were prepared from fish meat protein, as well as from horse meat protein, in the same manner as described before. Young rats were fed first on the diet consisting of:

Fish meat protein	15%	Starch	65%
Butter (ether soluble part)	15%	McCollum's salt mixture	5%
Alcohol extract of yeast	5%		

and let grow for 8 days. When the whole of the protein was replaced by each hydrolysate, the animals soon began to decline in body weights, and finally succumbed (Fig. IV, XV).

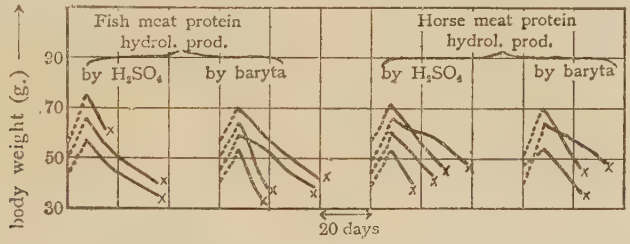


Fig. VI. Experiments with the acid or the baryta hydrolytic products.
- - - - - - fish meat protein.
——— hydrolytic product of protein.

IV. Experiments with the acid hydrolytic products supplemented with tryptophane.

In the first place, the tryptophane contents of the fish meat protein and its hydrolytic products were estimated according to Matsuyama and Mori's method[8], the tryptophane content of pure casein being assumed to be 1·5%

The results obtained were as follows:

Fish meat protein	1.24%	Acid hydrolytic products	0.00
Baryta hydrolytic products	1.78%		

From these results, it can be seen that tryptophane is completely decomposed by boiling with acid, but it is not destroyed, by treating with baryta. Intending now to decide whether the indispensable factor in the baryta hydrolytic product, which is lacking in the acid hydrolytic products, is tryptophane only the baryta hydrolytic products were fractionated by mercuric sulphate into two parts; i.e. the one, consisting solely of crystalline tryptohane and the other, the filtrate from $HgSO_4$-preciptate containing no tryptophane.

(8) Matsuya & Mori: J. Chem. Soc. Jap., **44** (1923), 377.

Mercury and sulphuric acid in the filtrate were removed by hydrogen sulphide and baryta respectively, the resulting filtrate was neutralized with dilute caustic soda, and then evaporated to dryness at lower temperature. From 200 g. of fish meat protein, 1.5 g. of crystalline tryptophane and 130 g. of the filtrate part were obtained.

Tryptophane obtained as above stated was optically inactive, and gave the following analytical result.

		N%
Subst. 3.750 mg.	0.439 c.c. N (765.7 mm, 21.5°)	13.67
3.435 mg.	0.400 c.c. N (765.8 mm, 22.0°)	13.56
Calculated for tryptophane:		13.72

Rats were fed first on fish meat protein and let grow for 8 days, then the protein was replaced by its acid hydrolysate, so the rats began to lose their body weights, owing to the lack of tryptophane. Then two rats were supplied with racemic tryptophane* to the extent of 3% of the acid hydrolysate, and to the other two rats the filtrate part of the baryta hydrolysate was given repalacing half the quantity of the acid hydrolysate. Hereupon, a sudden increase of body weight was resulted in the former case namely on the ration containing tryptophane. Rats grew normally from 64 g. and 65 g. up to 187 g. and 190 g. respectively in 70 days, while the rats receiving the latter ration lost their body weights remakably. To the remaining one, supplied with tryptophane, and grown vigorously for 38 days was given the baryta hydrolysate, replacing half the quantity of the acid hydrolysate, tryptophane being omitted. In this case also, the rat soon began to decline in body weights (Fig. V, XII, XIII, XIV). From these results, it may be seen that the filtrate part of the baryta hydrolysate has no supplementing effect on the acid hydrolysate, but, what should give us concern is that some toxic substance might contaminate the filtrate during the process of fractionation with mercuric sulphate, so the experiments were repeated with the following scheme:

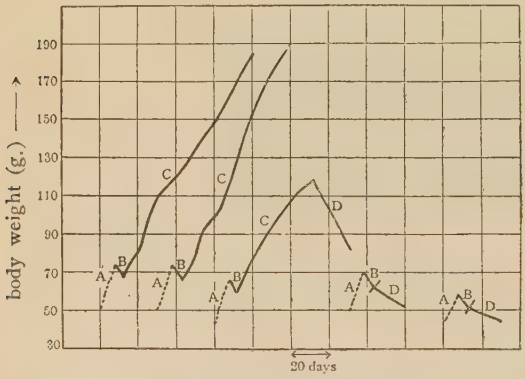


Fig. V. Experiments with acid hydrolytic product supplemented with tryptophane.
A: Fish meat protein; B: Acid hydrol. prod.
C: B. suppl. with tryptophane (3% of the acid hydrol. prod.)
D: B. suppl. with the filtrate part in the baryta hydrol. prod.

* 20 g. of racemic tryptophane was prepared from 2.5 kg. of casein by boiling with baryta.

Rats were fed first 20 days on the diet consisting of:

Acid hydrolytic products	7.5%	Butter (ether soluble part)	15.0%
Baryta hydrolytic products	7.5%	McCollum's salt mixture	5.0%
Starch	65.0%	Alcohol extract of yeast	5.0%

After 20 days, when the baryta hydrolytic products were replaced by its tryptophane-free filrate part, the growing rats soon began to loose their weights remarkable. By adding tryptophane (3% of the hydrolytic products) the rats revived suddenly and began to grow vigorously. After 20 days, the filtrate part was removed from the above diet but no effect on growth was observed and the rats continued to grow normally as before (Fig. VI). From these results, the author wishes to conclude that the acid hydrolytic products of protein, when supplemented with tryptophane, can replace the protein in diets completely.

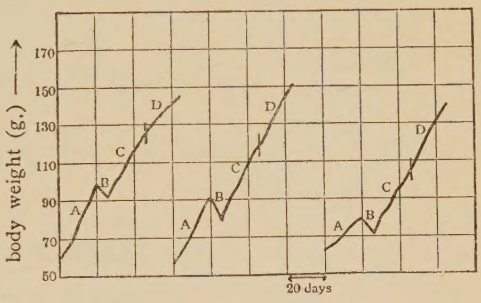


Fig. VI. Similar experiments as shown in Fig. II.

A: Mixture of the acid and the baryta hydrol. prod.

B: Acid hydrol. prod. and the filtrate part in the baryta hydrol. prod.

C: B. suppl. with tryptophane (3% of the hydrol. prod.)

D: Acid hydrol. prod. suppl. with 3% of tryptophane.

In conclusion, the author wishes to expess his deepest obligations to Prof. U. Suzuki for his kind directions throughout this work.

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